REMARKS

This Reply is responsive to the Office Action dated October 19, 2001. Entry of the foregoing and reconsideration on the merits pursuant to 37 CFR 1.116 is respectfully requested.

At the outset, applicants wish to thank the Examiner for the helpful advice given in the telephonic interview on December 3, 2001. The amendments entered herein are intended to reflect what was discussed in the interview, with the goal of expediting an allowance of the present application. Therefore, if the Examiner believes that any of the amendments presented do not effectively accomplish the applicants' intentions, the Examiner is urged to contact the undersigned to discuss alternative language that would facilitate an allowance in this application.

Also, in response to the Examiner's inquiry, applicants have not yet received a signed copy of the PTO-1449 form submitted August 14, 2001 indicating that the cited references have been considered.

The application has been amended as set forth above. In accordance for the new rules for amending applications set forth in 37 CFR 1.121, which took effect on March 1, 2001, a marked up version of the claims showing all amendments is attached hereto as an appendix. Specifically, claim 1 was amended to limit the claimed method to inhibiting expression of a target gene in a cell in vitro in order to pursue allowable subject matter as indicated in the Office Action (see page 3, lines 3-4). This limitation precludes the operation of dependent claims 12 and 17-20, which were therefore canceled.

Claim 1 was also amended to clarify that the RNA used in the claimed method is one that consists essentially of a double-stranded structure wherein the first and the second ribonucleotide sequences are separate complementary sequences. This amendment incorporates the limitation of claim 14 and precludes the operation of claim 13, both of which were therefore canceled.

Claim 1 was also amended to delete the phrase "stably anneal" which was rejected in the Office Action, and to substitute this phrase with the phrase "hybridize to each other to form said double-stranded structure," of which the Examiner preliminarily approved during the telephonic interview.

Claim 1 was also amended to include a positive process step, whereby it was specified that the double-stranded structure inhibits expression of the target gene. In addition, claim 1 was amended to delete reference to the lower length limit of 25 bases, which the Examiner

agreed was not necessary so long as applicants avoided indefinite language like "portion" or "fragment" of the targeted gene. Claim 10 was in turn amended to change the recited lower limit of 50 bases to 25 bases, in view of the cancellation of this limitation from claim 1.

Claims 15 and 16 were amended to depend on claim 1 in view of the incorporation of the limitation of claim 14 into amended claim 1.

Claim 22 was amended to limit the claimed method to inhibiting expression of a target gene in an invertebrate organism in order to pursue allowable subject matter as indicated in the Office Action (see page 3, lines 3-4; page 6, line 10; and page 7, line 4). This amendment incorporates the limitation of claim 25 and precludes the operation of dependent claims 23 and 24, which were therefore canceled.

Claim 22 was also amended to clarify that the RNA used in the method consists essentially of a double-stranded structure formed by two separate ribonucleic acid strands that hybridize to each other. Claim 22 was also amended to delete reference to the lower length limit of 25 bases, as the Examiner agreed during the telephonic interview that this limitation was unnecessary. Dependent claim 28 was therefore amended to replace the recited length limit of 50 bases with the value of 25 bases in view of the amendment to claim 22.

Claim 32 was amended for grammatical purposes.

Claim 39 was also amended to delete reference to the lower length limit of 25 bases. Claims 40-46 were canceled in favor of the amended claims presented above.

In addition, several new claims (47-51) were presented in order to pursue more narrowly drafted claims to methods of inhibiting the expression of a target gene in a vertebrate organism. In this regard, it is applicants' understanding (as discussed further in depth below) that the Examiner believes the problem with extending the scope of the claims to use in mammals and other vertebrate organisms lies in the general unpredictability of delivery methods. However, the new claims presented limit the claimed methods to specific forms of delivery for mammals that are supported by the specification and were well known in the art by the time this invention was filed.

For instance, new claim 47 is directed to a method of inhibiting expression of a target gene in a vertebrate organism wherein the RNA is introduced by directly injecting the RNA into the organism in the vicinity of the target cell such that the RNA is introduced into the target cell and thereby inhibits expression of the target gene. Support for this claim may be found in the specification at the very least at page 14, line 8, and page 26, lines 14-17. It was well known prior to the filing of this invention that direct injection of naked nucleic acids into

the muscle or skeletal muscle results in high levels of expression of injected reporter genes, via uptake of the nucleic acids into cells in the vicinity. See, e.g., U.S. Patent 5,580,589, enclosed herein. See also U.S. Patent No. 5,770,580, also enclosed, col. 3, lines 32-35.

As disclosed in the present invention, the ds-RNAs of the invention were shown to cross cellular boundaries following direct injection into *C. elegans*. See page 26, lines 14-17. Moreover, it is now known that RNA interference works in both mammalian and plant cells, as predicted in applicants' disclosure (please see the enclosed abstracts as well as the enclosed copy of the public announcement by Agy Therapeutics). Given that all the parameters of delivering nucleic acids to mammalian cells by direct injection were worked out prior to the filing of the present invention, there is no reason to believe that local delivery of the claimed dsRNAs by direct injection into a mammal could not be done as disclosed in the specification. Therefore, applicants respectfully request that the examiner consider allowing claim 47 along with the amended claims.

Similarly, claim 48 is directed to a method of inhibiting expression of a target gene in a vertebrate organism wherein the gene is expressed in a target cell that contacts a body cavity or interstitial space of said organism, wherein the dsRNA is introduced into the body cavity or interstitial space of said organism such that the RNA is introduced into the target cell and thereby inhibiting expression of the target gene. Support for this claim may be found at the very least at page 13, lines 30-31 and page 26, line 15. It was known at the time this invention was filed that cells associated with fluid spaces uptake naked nucleic acids that are injected into those fluid spaces. See U.S. Patent No. 5,770,580. Further, it has been known since at least 1986 that DNA that is injected into the intraperitoneal cavity of rats is taken up and expressed by the animal's tissues (see the enclosed abstract by Benvenisty and Reshef).

Applicants disclose in the specification that dsRNAs injected into the body cavity of *C. elegans* produces gene-specific inhibition in the surrounding tissues. See page 26, lines 15-20. And as mentioned above, it is now known that RNA interference works in both mammalian and plant cells, as predicted in applicants' disclosure (please see the enclosed abstracts as well as the enclosed copy of the public announcement by Agy Therapeutics). Given that all the parameters of delivering nucleic acids to mammalian cells by direct injection into fluid spaces and body cavities for local transfection of tissues were worked out prior to the filing of the present invention and it was known that cells associated with those spaces would uptake such nucleic acids, there is no reason to believe that local delivery of the claimed dsRNAs by delivery into a mammalian body cavity or interstitial space could not be

done as disclosed in the specification. Therefore, applicants respectfully request that the examiner consider allowing claim 48 along with the amended claims.

Similarly, claim 49 is directed to a method of inhibiting expression of a target gene in a vertebrate organism, wherein the gene is expressed in a target cell that is accessible by the digestive tract of said organism, by orally administering the RNA to the organism such that the RNA is introduced into the target cell and inhibits expression of the target gene. Support for this claim may be found at the very least at page 14, lines 1-4, and page 26, lines 22-25. Claims 50 and 51 are dependent on claim 49, and find support in original claims 19 and 20. The Examiner submits in the Office Action at page 5 that oral delivery of nucleic acids would not be effective in mammals because the RNA would be degraded by digestive enzymes. However, this is certainly not the case. U.S. Patent 6,225,290, enclosed herein, discloses oral administration of naked DNA to mammals to accomplish intestinal cell transformation. Although this patent issued after the present invention was filed, it confirms applicants' disclosure that oral administration of dsRNA to a mammal is a valid form of *in vivo* delivery. Indeed, the nematodes with which applicants worked produce digestive enzymes, and such enzymes did not interfere with the function of the dsRNAs fed to such nematodes. See enclosed abstracts by Joshua, Fukushige et al., Kennedy et al., and Aamodt et al. Therefore, there is no reason to believe then that cells associated with the digestive tract of a mammal could not be targeted by the claimed dsRNAs using the methods disclosed by the specification. Accordingly, applicants respectfully request that the Examiner consider allowing claims 49-51 along with the amended claims.

No new matter has been added by any of the amended or new claims presented above. Applicants respectfully request entry and allowance of all the claims presented.

Turning now to the Office Action, claims 1-6, 10-23, 27-35 and 41-46 were rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for methods of inhibiting a target gene using a double-stranded RNA in a nematode or *in vitro*, allegedly fails to provide enablement for methods of inhibiting a target gene using a double-stranded RNA in any organism *in vivo*, or any "whole" organism. Applicants do not agree with the rejection, but nevertheless, it appears that the rejection may be rendered moot by the amendments and new claims submitted above.

For instance, claim 1 was amended to limit the claimed method to inhibiting expression of a target gene using dsRNA in a cell *in vitro*. Therefore, the claim does not encompass a method of targeting a whole organism, and is enabled as acknowledged in the

Office Action. Claims 2-11, 15, 16 and 21 are dependent either directly or indirectly on claim 1 and therefore incorporate all the limitations therein. Therefore, these dependent claims would also be enabled.

Likewise, claim 22 was amended to limit the claimed method to inhibiting expression of a target gene using dsRNA in a cell in an invertebrate organism. The Office Action acknowledges enablement as to invertebrate organisms (see page 6, lines 6-11), therefore, the rejection as to claim 22 as amended is moot. Claims 26-35 are dependent either directly or indirectly on claim 1 and therefore incorporate all the limitations therein. Therefore, these dependent claims would also be enabled.

As applicants understand, the main issue with the scope of the claims encompassing the *in vivo* targeting of genes in whole organisms such as mammals is the lack of predictability in delivering nucleic acids to a target cell of interest. For instance, as stated on page 4 of the Office Action, at the beginning of the last paragraph, "Methods of inhibiting gene expression using nucleic acids *in vivo* (whole organism) are highly unpredictable, mainly due to issues of how to specifically deliver a nucleic acid molecule or vector to a target cell at a concentration effective to result in a desired effect, and in the case of gene therapy, the determination of target cell-specific vectors and promoters to achieve and maintain expression of the gene."

Applicants respectfully note that new claims to *in vivo* targeting are presented above, but according to the reasons set forth in the Office Action, they should not be subjected to the present rejection under 35 U.S.C. §112, first paragraph. Indeed, the basis for the rejection as discussed in the above passage is that a specific method for delivering an effective concentration must be provided. New claims 47-49 recite specific delivery methods that are disclosed in the specification. Furthermore, the prior art had already established the parameters for effectively accomplishing these methods before the present invention was filed. Because applicants need not teach what is known in the art, the specification must be considered enabling for specific delivery methods that were known to be effective at the time.

For instance, new claim 47 is directed to a method of inhibiting expression of a target gene in a vertebrate organism wherein the RNA is introduced by directly injecting the RNA into the organism in the vicinity of the target cell such that the RNA is introduced into the target cell and thereby inhibits expression of the target gene. It was well known prior to the filing of this invention that direct injection of naked nucleic acids into the muscle or skeletal muscle results in high levels of expression of injected reporter genes, via uptake of the

nucleic acids into cells in the vicinity. See, e.g., U.S. Patent 5,580,589, enclosed herein. See also U.S. Patent No. 5,770,580, also enclosed, col. 3, lines 32-35. Given that all the parameters of delivering nucleic acids to mammalian cells by direct injection were worked out prior to the filing of the present invention, there is no reason to believe that local delivery of the claimed dsRNAs by direct injection into a mammal could not be done as disclosed in the specification.

Similarly, claim 48 is directed to a method of inhibiting expression of a target gene in a vertebrate organism wherein the gene is expressed in a target cell that contacts a body cavity or interstitial space of said organism, wherein the dsRNA is introduced into the body cavity or interstitial space of said organism such that the RNA is introduced into the target cell and thereby inhibiting expression of the target gene. It was known at the time this invention was filed that cells associated with fluid spaces uptake naked nucleic acids that are injected into those fluid spaces. See U.S. Patent No. 5,770,580. Further, it has been known since at least 1986 that DNA that is injected into the intraperitoneal cavity of rats is taken up and expressed by the animal's tissues (see the enclosed abstract by Benvenisty and Reshef). Therefore, there is no reason to believe that local delivery of the claimed dsRNAs by delivery into a mammalian body cavity or interstitial space could not be done as disclosed in the specification.

Similarly, claim 49 is directed to a method of inhibiting expression of a target gene in a vertebrate organism, wherein the gene is expressed in a target cell that is contacted by the digestive tract of said organism, by orally administering the RNA to the organism such that the RNA is introduced into the target cell and inhibits expression of the target gene. The Examiner submits in the Office Action at page 5 that oral delivery of nucleic acids would not be predicted to be effective because the RNA would be degraded by digestive enzymes. However, this is certainly not the case. U.S. Patent 6,225,290, enclosed herein, proves applicants' prediction that oral administration of naked nucleic acids to mammals may be used to accomplish intestinal cell transformation. Furthermore, the nematodes with which applicants worked produce digestive enzymes, and such enzymes did not interfere with the function of the dsRNAs fed to such nematodes. See enclosed abstracts by Joshua, Fukushige et al., Kennedy et al., and Aamodt et al. Therefore, there is every reason to believe that cells associated with the digestive tract of a mammal could be targeted by the claimed dsRNAs using the methods disclosed by the specification with negligible experimentation.

The Office Action has already acknowledged that targeting any cell *in vitro* using the claimed methodology - including mammalian cells - is enabled by the specification. Indeed, abstracts attached hereto confirm applicants' prediction, that RNA interference operates in a wide diversity of cell types. For instance, Billy and colleagues teach that double-stranded (ds) RNA induces sequence-specific inhibition of gene expression in mouse embryonal carcinoma (EC) P19 and F9 cells and mouse embryonic stem cells. Yang and colleagues disclose the use of RNA interference in several mammalian cells, either by in situ production of dsRNA from transient transfection of a plasmid harboring an inverted repeat or by direct transfection of dsRNA made by in vitro transcription. Tenllado and Diaz-Ruiz, Jr. show that dsRNA derived from viral sequences can interfere with virus infection in a sequence-specific manner by directly delivering dsRNA to leaf cells either by mechanical inoculation or via an Agrobacterium-mediated transient-expression assay. Escobar and colleagues demonstrate RNA interference-mediated oncogene silencing in transgenic Arabidopsis thaliana and Lycopersicon esculentum plants. Levin and colleagues show that expressing both antisense and sense RNA together is an effective means of inactivating reporter and viral genes in plants. And Schweizer and colleagues show sequence-specific inhibition of gene expression in single epidermal cells of maize, barley or wheat following particle bombardment with dsRNA.

Given that RNA interference operates in a wide diversity of cell types and specific methods of delivering nucleic acids effectively *in vivo* were known prior to the present invention, applicants are entitled to claims of *in vivo* delivery methods that are supported by their disclosure. Indeed, applicants demonstrate *in vivo* delivery in *C. elegans* by direct injection, and show that the injected dsRNA molecules cross cellular boundaries and mediate sequence specific gene inhibition in the cells in the vicinity of the injection. Claim 47 fairly reflects this showing, as it would be entirely expected that the same direct delivery injection would work in vertebrates *in vivo*, given the knowledge existing in the art at the time (e.g., U.S. Patent 5,580,859).

Applicants also demonstrate that injecting dsRNA into a body cavity of *C. elegans* results in sequence-specific inhibition of the cells in the surrounding tissues. It would be entirely expected that this delivery method would also work in mammals given the knowledge in the art at the time of filing, and claim 48 fairly reflects such an *in vivo* method of delivery.

Applicants also demonstrate that feeding dsRNA to *C. elegans* results in the sequence-specific inhibition of target gene expression, despite the presence of digestive enzymes. Therefore, it would be entirely expected that a similar made of delivery would work for mammals, particularly given the disclosure of U.S. Patent 6,225,290 which has since issued and confirms applicants' predictions.

Thus, in contrast to what is alleged in the Office Action, the specific delivery methods disclosed in the specification would facilitate sequence-specific inhibition of gene expression in whole vertebrate organisms. Applicants respectfully request that the rejection not be applied to new claims 47-51, as these claims are drafted to read only on these localized forms of *in vivo* delivery. Further, applicants respectfully request withdrawal of the rejection as to the amended claims.

Next, claims 1-6, 8-11, 13, 17, 18, 22, 23, 25, 26, 28, 30, 31, 40, 41 and 43-46 were rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Agarwal (WO 94/01550). Without agreeing with the rejection, applicants note that the claims have been amended to refer to the use of double stranded RNAs consisting essentially of two separate strands that are hybridized together. Agarwal discloses only single stranded polynucleotides that are self-complementary and fold back onto themselves, thereby forming a loop or hairpin that protects the single strand from degradation. In this regard, the hairpin or loop in the RNA constructs of Agarwal is essential to confer the enhanced stability. Thus, Agarwal fails to teach a structure consisting essentially of two complementary strands, and Agarwal does not anticipate the claims as amended.

Claim 39 was rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Agarwal. Without agreeing with the rejection, applicants note that claim 39 has also been amended to refer to the use of double stranded RNAs consisting essentially of two separate strands that are hybridized together. Agarwal discloses only single stranded polynucleotides that are self-complementary and fold back onto themselves, and the hairpin or loop thereby formed is essential to provide enhanced stability for the disclosed polynucleotides. Therefore, Agarwal does not suggest the use of a double stranded RNA having two separate strands, and does not render obvious the kit as recited in claim 39.

Claims 1-6, 8-21, 39-41 and 43-46 were rejected under 35 U.S.C. §§112, first and second paragraphs because of the phrase "stably annealed." Specifically, the Examiner believed that this phrase raises clarity issues under the second paragraph of §112, and was not supported by the specification as filed. Without necessarily agreeing with the rejection,

applicants note that the claims have been amended to delete the rejected phrase. Therefore, these rejections are now moot.

Claim 21 was rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over the combination of Agarwal and Mercola. The basis for the rejection was that Mercola teaches the expression of antisense RNA using an expression vector as recited in claim 21, and that an antisense RNA that folds back on itself as disclosed in Agarwal allegedly taught all the limitations recited in the base claim. Without agreeing with the rejection, applicants note that the base claim has been amended such that it is directed to the use of double stranded RNAs consisting essentially of two separate strands, which is not anticipated or rendered obvious by Agarwal. Because claim 21 incorporates the limitations of the base claim and Agarwal is therefore no longer applicable, this rejection is rendered moot.

Finally, applicants note that claim 7, which is dependent on claim 1 and limits the claimed method to inhibiting target gene expression in a plant cell, has not been canceled. In this regard, applicants note that claim 7 is a species claim that incorporates all the limitations of the base claim and may therefore be rejoined with the elected invention upon the indication of allowable subject matter, presuming of course that the claim satisfies the requirements of 35 U.S.C. §§101, 102, 103 and 112. See MPEP 809, Claims Linking Distinct Inventions. Seeing as RNA interference has been shown to work in plant cells (as provided in the attached abstracts), and seeing as the base claim has been limited to *in vitro* methods, it would seem that there are no patentability issues that would preclude claim 7 being rejoined with the subject application upon allowance.

All issues raised by the Office Action dated October 19, 2001 have been addressed in this Reply. Accordingly, a Notice of Allowance is next in order. If the Examiner has any further issues to raise regarding the subject application or any additional suggestions regarding the language of the claims, applicants respectfully request that she contact the undersigned so that such issues may be addressed expeditiously.

All objections and rejections having been addressed, it is respectfully submitted that the present application is in a condition for allowance and a Notice to that effect is earnestly solicited.

Respectfully submitted,

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